

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

(SEE ATTACHED PAGES)

8. Additional Requirements:

Nil

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9. Changes in Personnel with Biographical Sketches of new Personnel (append)

New personnel: Dr. Gaetano Giraldo (See biographical sketch attached)

10. Publications or Papers in Press resulting from the Project or closely related work

(SEE ATTACHED LIST)

### C. Additional experiments.

In addition to the original experimental design, investigations are being carried out on:

Mitotic cell activity in the mouse lung and immunological approaches of INH and h.s. induced lung tumours in mice.

a. Preliminary studies on the mitotic cell turnover in the mouse lung.

This experiment is being carried out with special fixation and staining techniques in lung of lung tumour induction susceptible (BALB/c/Cb/Se) and resistant (CBA/Cb/Se and C57BL/Cb/Se) mice, treated and non treated and in different hormonal states.

This should allow us to observe the early cell changes and the cells chiefly involved in the regenerative process. Differences between the three groups of mice - treated, non treated and in different hormonal states - could be of significant interest.

b. Immunological approaches to chemically induced lung tumours in mice.

(i) Attempts to show whether lung tumours obtained with INH and h.s. are merely accelerated spontaneous tumours or whether they are induced.

It has been shown that chemically induced tumours develop specific antigens, whereas spontaneous tumours do not.

Moreover, whereas virus induced tumours usually carry antigens specific to the virus and therefore common to every tumour induced by that virus, the tumour specific antigens of chemically induced tumours differ from tumour to tumour.

The application of this approach to INH and h.s. induced lung tumours could proceed along the following lines: part of a large lung adenoma could be transplanted into mice of the same strain; when it grew out, a part of it could be excised and fragments implanted into other mice, and removed when they started to grow out. The ability of further fragments of the same tumour, or of measured numbers of cells from it, to be rejected by the immunized mice would demonstrate the antigenicity of these tumours.

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D 7. Changes or Additions to Experimental Design and Procedures:

A. Spontaneous and INH and h.s. induced pulmonary tumours in the mouse in relation to adrenal hormones.

a. Incidence of spontaneous and induced pulmonary tumours in susceptible and resistant mice.

There have been no changes made to these experiments, which have been described in the Application and in Progress Report no. 1.

b. Incidence of spontaneous pulmonary tumours in susceptible adrenalectomized mice into which the adrenals of resistant mice have been grafted, and vice versa.

At the beginning of this experiment (see Application and Progress Report no. 1) A/Lac/Se and C57BL/Cb/Se mice, respectively susceptible and resistant to lung tumour induction, were used.

To assure that the adrenals take in the host it is necessary to treat the test mice at birth with lymphocytes of the donor mouse.

The administration (intravenous or intraperitoneal) of lymphocytes between these two substrains, however, is badly tolerated: none of the C57BL/Cb/Se mice treated with A/Lac/Se lymphocytes lived beyond a few days; the same thing happened when A/Lac/Se mice were the tests and the C57/Cb/Se the donors. None of the technical procedures used was effective in preventing the death of the test mice. For this reason, the C57BL/Cb/Se mice were replaced by CBA/Cb/Se. The tolerance between CBA/Cb/Se and A/Lac/Se mice has proved good, provided the test mice are treated within a few hours of birth. CBA/Cb/Se mice are considered resistant to lung tumour induction and therefore may replace the C57BL/Cb/Se.

Several newborn mice of the two substrains have already been treated, and survival is good; at 8 weeks they will be adrenalectomized, and have the adrenals of the lymphocyte donor grafted into them; they will be examined at natural death. This has already been described in the Application and in Progress Report no. 1.

c. Pulmonary tumorigenesis by INH and h.s. in susceptible adrenalectomized mice into which the adrenals of resistant mice have been grafted, and vice versa.

These experiments will be carried out as described in the Application, with CBA/Cb/Se mice replacing the C57BL/Cb/Se.

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## 11. Budget (for coming year) (October 1st, 1967 to September 30th, 1968)

## A. Salaries (Personnel by names or category)

## Professional

Dr. Rodolfo Ribacchi  
Dr. Emilio Buccarelli  
Dr. Gaetano Giraldo

## Technical

Histological technician  
Animal caretaker

% time	Amount
50%	
50%	
50%	

Sub-Total

\$	R	R
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## B. Consumable Supplies (list by categories)

Animal food and sawdust	\$ 1,000
Chemical drugs	500
Staining materials	250
Surgical instruments	150
Glassware	100

Sub-Total

\$ 3,000	3,000
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## C. Other Expenses (itemize)

Record cards, registers, labels

Sub-Total

300	300
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## D. Permanent Equipment (itemize)

800 animal cages

1,700	
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1,700	
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## E. Overhead (15% of A+B+C)

Total	15,000
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\$ 11,600	
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It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature hans lewe Director of Project

Telephone \_\_\_\_\_

Signature hans lewe Business Officer of the Institution

Telephone \_\_\_\_\_

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10. Publications or Papers in Press resulting from the Project or closely related work.

1. Ribacchi, R. and Giraldo, G.: Sui fattori intrinseci della cancerogenesi polmonare del topo: effetti della tinectomia neonatale. Lav. Anat. Pat. Perugia 26, 127-136, 1966.
2. Severi, L. and Biancifiori, C.: Hepatic carcinogenesis in CBA/Cb/Se mice and Cb/Se rats by hydrazine sulphate. International Conference on Hepatic Regeneration, Montecatini, 29th-30th October, 1966. (In press)
3. Severi, L. and Biancifiori, C.: Hepatic carcinogenesis in CBA mice and Cb rats by isonicotinic acid hydrazide and hydrazine sulphate. Symposium on Hepatomas, Philadelphia, 19th-20th May, 1967. (The paper will be forwarded to the Journal of the National Cancer Institute for publication)

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This experiment will have to be repeated several times. It should, however, be confirmed also by other experiments, with the aim of showing that the participation of a virus in the development of the pulmonary tumour can be excluded, but this will not show that a hormonal factor is not involved. The latter is an internal component which probably does not act by itself but, in favourable conditions, might allow the action of INH or h.s. or of any other carcinogen.

- (ii) Attempts to influence the growth of urethane induced primary lung tumours in BALB/c mice by stimulation of immunocompetent cells of the host.

Previous work done in this laboratory (1965, 1966) has shown that (the depression of immunocompetent cells obtained by) neonatal thymectomy potentiates the oncogenic effect of a single dose of urethane in BALB/c mice. In fact, in thymectomized mice lung tumours develop earlier, are more numerous, grow to a greater size, metastasize more frequently than in controls treated with the same dose of urethane, and they only rarely show foci of necrosis and/or fibrosis.

Theoretically, a stimulation of immunocompetent cells in mice treated with a single dose of urethane could reduce the oncogenic effect of the chemical compound. Following this approach, BALB/c mice inoculated with urethane will be treated with incomplete and complete Friend's adjuvant, with urethane-induced secondary BALB/c lung tumour grafts, and with a cell-free homogenate of the same tumour.

8. (See printed form)

9. Biographical Sketch of Dr. Gaetano Giraldo.

Title: Assistant scientist, Division of Cancer Research,  
University of Perugia

Place of Birth: Naples, Italy Present Citizenship: Italian  
Sex: Male

Education and Experience: Dr. Giraldo graduated M.D. from the University of Naples in 1962. He is a staff member of the Institute of Pathological Anatomy and Division of Cancer Research, University of Perugia, from 1963.

Dr. Giraldo has published 17 papers on various subjects.

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d. Incidence of spontaneous pulmonary tumours in intact and in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F<sub>1</sub> hybrid mice.

No change has been made to the experiment as described in the Application but, as mentioned in Progress Report no. 1, (BALB/c/Cb/Se x C57BL/Cb/Se)F<sub>1</sub> hybrid mice will also be used.

e. Incidence of spontaneous pulmonary tumours in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F<sub>1</sub> mice into which the adrenals of the parents have been grafted.

No changes: the experiments will be carried out as described in the Application. In these experiments as well (BALB/c/Cb/Se x C57BL/Cb/Se)F<sub>1</sub> hybrid mice will also be used.

f. Pulmonary tumorigenesis by INH and h.s. in intact and in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F<sub>1</sub> hybrid mice.

Also in these experiments, in addition to the (C57BL/Cb/Se x A/Lac/Se)F<sub>1</sub> hybrid mice (as shown in the Application), (BALB/c/Cb/Se x C57BL/Cb/Se)F<sub>1</sub> hybrid mice will be used (as shown in Progress Report no. 1).

g. Pulmonary tumorigenesis by INH and h.s. in adrenalectomized (C57BL/Cb/Se x A/Lac/Se)F<sub>1</sub> hybrid mice into which the adrenals of the parents have been grafted.

These experiments will be carried out as previously described in the Application; (BALB/c/Cb/Se x C57BL/Cb/Se)F<sub>1</sub> hybrid mice will be added.

If it seems of use, (CBA/Cb/Se x A/Lac/Se)F<sub>1</sub> hybrid mice will be added to the experiments described in paragraphs d,e,f,g.

These different types of hybrids will be used because they are the product of substrains of mice in each of which the spontaneous incidence and the susceptibility to lung tumour induction are different. If these characteristics in the adrenalectomized hybrid test mice are the same as in the parent donor of the adrenals, the influence of these glands in lung carcinogenesis could be hypothesized.

B. Spontaneous and INH and h.s. induced pulmonary tumours in the mouse in relation to thyroid hormones.

No changes made to the experiments as described in a,b,c,d of the Application.

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current	Title of Project	Source	Amount	Duration
	Hormonal factor(s) in pulmonary carcinogenesis (by hydrazine). (Severi, L. and Biancifiori, G.)	The Anna Fuller Fund, New Haven, Connecticut	\$ 5,000 per year	Dec. 1965 to Nov. 1968
	Lung tumorigenesis by isoniazid (INH), its metabolite hydrazine sulphate and derivatives of hydrazine. (Biancifiori, G.)	The Council for Tobacco Research, 633 Third Avenue, New York N.Y. 10017	\$11,100 10,100	Ap. 1966 to Mar. 1967 Mar. 1968
Pending				

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BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

NO. 567RL

Activated: 10/1/66

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE  
NEW YORK, N.Y. 10017

COMMITTEE:

Dr. Little, Chm.  
Dr. Sommers  
Dr. Reimann  
Dr. Loosli

Application For Renewal of Research Grant

First  Second

Date: 19th July, 1967

1. Name of Investigator(s): (include title and degree) Prof. Lucio Severi, M.D. Principal Investigator; Cesare Biancifiori, M.D. Co-Principal Investigator; Rodolfo Ribacchi, M.D. Assistant; Emilio Bucciarelli, M.D. Assistant;
2. Institution & Address: Gaetano Giraldo, M.D. Assistant

Division of Cancer Research, University of Perugia  
P.O. Box 327 PERUGIA ITALY

3. Short Title of Project:  
An Approach to the Study of Internal Factors in Lung Carcinogenesis.  
Influence of Hormones.

4. Proposed Renewal Starting Date: (Anniversary or other) October 1st, 1967

5. Discuss any Important Changes or Additions to Objectives or Specific Aims: There have been no basic changes made to the objectives proposed in the Application of 2nd August, 1966 and in the Semiannual Progress Report no. 1 forwarded in April, 1967 to The Council for Tobacco Research.

The observation of adrenal cortical damage in carcinogenesis by hydrazine/isonicotinic acid hydrazide (INH) seems a profitable line of investigation. In lung cancer patients changes in endocrine glands have been described. This is still the starting point for study on whether any internal factor exists in lung cancer.

- The aims of this research, therefore, remain chiefly:
- A. To study whether the adrenals are involved in spontaneous and INH and hydrazine sulphate (h.s.) induced pulmonary tumours in mice
  - B. To study whether the thyroid is involved in spontaneous and INH and h.s. induced pulmonary tumours in mice.

6. Give a Brief Statement of your Working Hypothesis if altered or modified:

There have been no changes made to the working hypothesis.

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